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The use of NaOH as transfer solution of DNA onto nylon membrane decreases the hybridization efficiency

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Recently a protocol for rapid DNA transfer (Southern blotting) to nylon membranes using sodium hydroxyde as the transfer solvent, has been described (1). We show here that, although the amount of bound DNA is indeed higher with a short alkaline transfer, transfer in ammonium acetate buffer (NH_4Ac) (2) and binding of DNA on membrane by conventional baking gives upon subsequent hybridization a five to ten fold better sensitivity. Two DNA preparations, one labelled, one unlabelled, to estimate respectively the binding and the hybridization efficiency were electrophoresed side by side on 1% agarose gels. All gels were then soaked in 0,25 N HCl 2x15 min. before transfer of the DNA to Zeta probe nylon membrane (Bio-Rad). For NaOH transfer the DNA was directly transferred in 0,4 N NaOH as described (1). The other gels were equilibrated twice in 0,5 N NaOH, 1,5 M NaCl for 20 minutes, twice in 1 M NH4Ac, 0,02 N NaOH for 30 minutes, before transfer of the DNA in 1 M NH4Ac, 0,02 N NaOH. After 24 hours of prehybridization, the DNA from the six membranes were incubated with the same amount (10^6 cpm/ml, $2x10^8$ cpm/µg) of nick translated p86 DNA for 24 hours in 50% formamide as described (3). The membranes were then washed three times 30 minutes with the last wash in 0.1 x SSC, 0.1% SDS at 65°C and autoradiographied. The comparison of these autoradiographies leads to three conclusions : 1) baking has no effect on the signal obtained (binding and hybridization) to DNA transferred in NaOH, but increase about two fold the retention and several fold the hybridization of the DNA transferred in NH4Ac. 2) with a short (4 hours) transfer there is more DNA bound to the membranes with alkaline transfer as judged by the intensity of the signal given by the labeled DNA. 3) after longer (16 hours) transfers, the amount of DNA on the membrane after the two types of transfer tend to become equal. However, the probe hybridizes about ten times better with NH4Ac than NaOH transferred DNA leading to a drastic increase in sensitivity. The same results were obtained with transfer of genomic DNA and visualization of a single copy gene (not shown).

REFERENCES : 1. Reed K.C. and Mann D.A. (1985) Nucl. Acids Res. 13 : 7207-7221. 2. Smith G.E. and Summers M.D. (1980) Anal. Biochem. 109 : 123-129. 3. Maniatis T., Fritsch E.F. and Sambrook J. (1982) Molecular Cloning : A laboratory Manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.

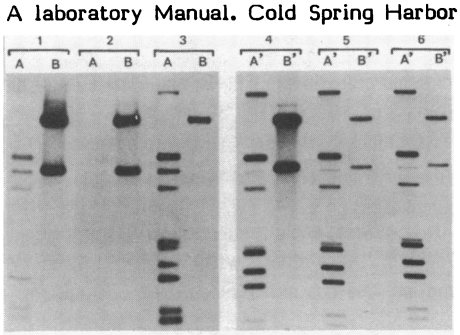

Fig. : NaOH transfer decreases the DNA hybridization capacity. A : 8.10^4 and A' 5.10^4 cpm/slot of ^{32}P end labeled HindIII and EcoRI cut, lambda DNA fragments. B : 250 and B' 150 ng/slot of cold p86 plasmid EcoRI DNA (8.6 and 4.3 kb) fragments.

	Transfer	baking (2 h, 80°C)
1 :	4 hours NH_4Ac	yes
2 :	4 hours NH_4Ac	no
3 :	4 hours NaOH	no
4 :	16 hours NH_4Ac	yes
5 :	16 hours NAOH	yes
6 :	16 hours NAOH	no

Gels were autoradiographed for 30' at room temperature.